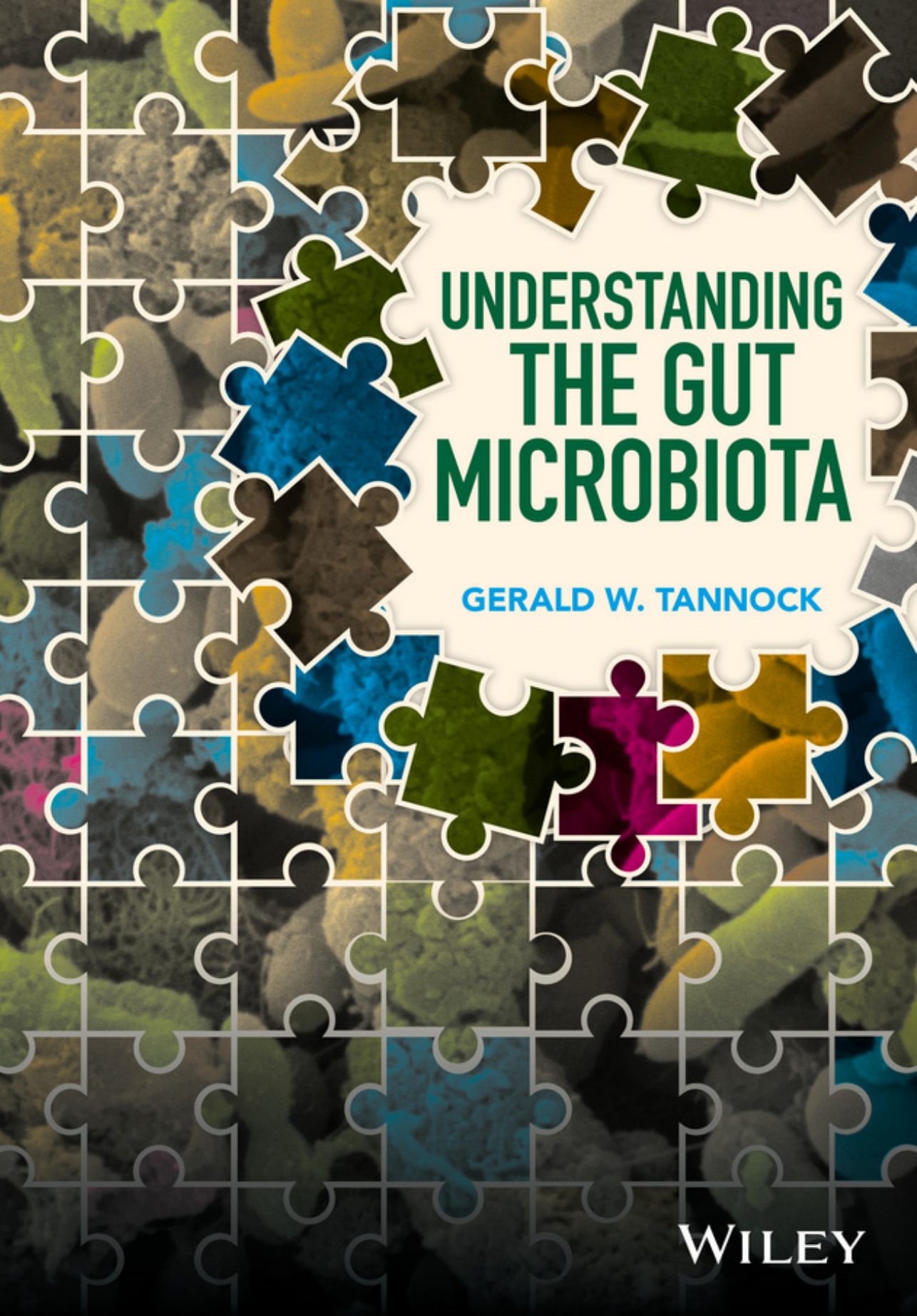
UNDERSTANDING
THE GUT
MICROBIOTA
GERALD W. TANNOCK
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Understanding the Gut Microbiota

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Gerald W. Tannock

Department of Microbiology and Immunology
University of Otago
Dunedin, New Zealand

WILEY

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Contents

Preface

Little more than 25 years ago, 'Big Biology' began with the formation of the Human Genome Project (HGP). This audacious scientific enterprise resulted in the development of advanced DNA sequencing technology, as well as methods for analyzing the large amounts of data this produced. The HGP was quickly followed by application of this technology and methodology to other biological topics, including the microbial communities that inhabit diverse ecosystems – these were evident, but their membership had not yet been deeply explored. The microbial community (microbiota) of the large intestine (colon) of humans was targeted as a focus of investigation using nucleic acid-based methodology because much of the community was then considered unculturable in the laboratory. Moreover, sequencing of the DNA extracted from human feces could reveal the biochemical capacity of the microbes as represented by their collective genomes (metagenome). Thus, data obtained from high-throughput DNA sequencing and bioinformatic analysis provided descriptions of the kinds of microbe (phylogeny) present in feces, as well as of the activities (functions) their DNA encoded. Putting phylogeny and metagenomic information together provided a description of the fecal microbiome. Big Biology projects like the Human Microbiome Project encouraged collaboration between scientists from various disciplines, and the formation of teams of investigators to study a common topic became part of a new way of doing science.

I have researched the microbiota of the large bowel of humans for more than 40 years, and I continue to actively research bowel bacteria, so this book provides a long-term perspective on our knowledge of this high-profile and fast-moving topic. Building on general ecological principles, the book aims to help the reader understand how the microbiota is formed, how it works, and what its consequences are for humans. It focuses on conceptual progress made from studies of the human bowel microbiota. Where appropriate, it draws on knowledge obtained from other animal species to provide conceptual enlightenment, but it is essentially a book about humans and their bowel microbes. It recommends particular research approaches to fill knowledge gaps, so that fundamental ecological theory and information about the microbiota can be translated into benefits for human health. The relationship between food for humans and resulting food for bowel bacteria emerges as an important topic for consideration.

Hopefully, the next 10 years will see the growth of further new ways of doing microbiota science. Bowel bacteria can be cultured together under special conditions in the laboratory, enabling the ecology of microbiotas to be explained mechanistically rather than descriptively. A combination of cultured bacteria, nucleic acid methodologies, chemical analyses, and bacterial physiology should see a continued flowering of interest in what bowel bacteria do and how they do it, and translation of this knowledge to help humans. We can pluck the fruits of Big Biology and use them in support of detailed studies of the microbial players in the bowel ecosystem: Micro Biology. This kind of work is open to even individual researchers and does not necessarily require the support of large financial resources. We must keep at the front of our minds that our aim in this research is to understand bowel bacteria. I hope that this book will help you to achieve that goal.

Gerald Tannock

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I extend my thanks to all those who have supported my endeavors in attempting to understand bowel bacteria over several decades. I have enjoyed many wonderful collaborations and friendships, and benefited from the intelligence, enthusiasm, and dedication of talented students, postdocs, and technicians. Thanks to Robbie McPhee, who prepared illustrations, and for the support of the folk at Wiley during the preparation of this book for publication. I wrote this book while a recipient of a James Cook Research Fellowship awarded by the Royal Society of New Zealand.

1

Introduction

This book is about the collection of microbes that inhabit the human colon. I became interested in the lives of gut bacteria when an undergraduate student at the University of Otago in Dunedin, New Zealand. During university vacations, I was employed as a "supernumerary" in the Department of Agriculture Veterinary Diagnostic Laboratory, Invermay Agricultural Centre, which is close to Dunedin. Not always rushed off my feet with laboratory work, I enjoyed the opportunity to occasionally browse the library literature, especially new journal articles. One day, I stumbled upon an article by René Dubos and his colleagues at Rockefeller University, New York on the subject of bacteria in the gut of mice. I found that there was a whole series of papers[1–5] from this group in the *Journal of Experimental Medicine*, and that some of them described experiments that used germfree animals. The articles really excited me, and led me to suggest a fourth-year Honors project on lactobacilli and porcine stomachs to Sandy Smith (who later became my PhD supervisor). I soon realized that the Rockefeller research was the first application of "microbial ecology thinking" to gut bacteriology.

One of the authors of the Rockefeller papers was Dwayne C. Savage. A particular paper,[5] of which he was first author, described the association of bacteria with epithelial and mucosal surfaces in the gut of mice. It so bowled me over that eventually I became a Fogarty International Postdoctoral Fellow in Dwayne's laboratory, first in Texas and then in Illinois. Dwayne's excellent mentoring set me on the career-long path of microbiota research that I have enjoyed ever since.

One of the thrilling sites that I saw in histological sections in the laboratory during my years in the United States was a layer of lactobacilli colonizing the epithelial surface of part of the murine stomach. It led me to spend much time in subsequent years investigating, with the help of a unique colony of *Lactobacillus*-free mice and some splendid laboratory associates, how a particular strain of *Lactobacillus reuteri* manages to live in the anterior gut of mice.

In 2001, Dwayne wrote a fine article for *Current Issues in Intestinal Microbiology* entitled "Microbial Biota of the Human Intestine: A Tribute to Some Pioneering Scientists."[6] It is worth reproducing the Abstract here, because it reminds us that much excellent research concerning gut bacteria was accomplished as long ago as the 1960s. It gives us some perspective on the topic:

> Research on the indigenous intestinal microbiota of man was initiated well before the end of the 19th Century. The work continued at a slow but steady

Understanding the Gut Microbiota, First Edition. Gerald W. Tannock.

> pace throughout the first half of the 20th Century. Findings from the effort had little impact on medicine and other aspects of human biology, however, until the 6th decade of the 20th Century. During that decade, research in the area was begun by eight groups of investigators, each of which was led by one or two senior scientists with great experimental talent, creativity and foresight. Their findings added new dimension to knowledge of the microbiota and initiated an explosion of interest in research in the field that has continued to the present day. The research of the groups during the 1960's is described in this review as a tribute to the senior scientists who had such critical impact on this important field of study.

Dwayne first pays tribute to Theodore Escherich,[7] who, more than 100 years ago, recognized, "At a time when microbiologic research has gained us so many laurels by following the research methods of Koch into the regions of the etiology and pathology of infectious diseases, it would appear to be a pointless and doubtful exercise to examine and disentangle the apparently randomly appearing bacteria in normal feces and the intestinal tract, a situation that seems controlled by a thousand coincidences. If I have nevertheless devoted myself now for a year virtually exclusively to this special study, it was with the conviction that the accurate knowledge of these conditions is essential, for the understanding of not only the physiology of digestion, but also the pathology and therapy of microbial intestinal diseases." He then records the contribution of Theodore Rosebury,[8] who provided a summary of the research undertaken from the 19th century through to the 1960s in his book, *Microorganisms Indigenous to Man*. The remainder of the review describes the eight groups of scientists whose work in the 1960s transformed microbiological and medical attitudes to the "normal flora" of the human gut. I, too, will list these heroes of gut microbiota research and summarize their contribution to the field – it is important to remember that we are "dwarfs standing on the shoulders of giants," and that our ability to understand a topic more deeply than our predecessors is not because we are smarter, but because we are lifted up by, and building on, their achievements:

- *B. S. Drasar and colleagues (UK).*[9] The association of bowel bacteria with certain diseases, the role of pH and peristalsis as regulatory factors in bowel ecology.
- *René Dubos, Russell Schaedler, Dwayne Savage, and colleagues (USA).*[10,11] Development of probably the first specific-pathogen-free (SPF) mouse colony, culture of previously unknown gut bacteria, impact of gut commensals on host physiology, association of bacteria with gut surfaces, a "microbial ecological" view of the gut ecosystem (symbiosis, biological succession).
- *Sherwood Gorbach and colleagues (USA).*[12] Sampling gastric and small-bowel contents, culture of commensals from these sites, investigation of diarrheal diseases, proponent of probiotics.
- *Bengt Gustaffson, Tore Midvedt, and colleagues (Sweden).*[13] Gnotobiotic animal experiments, description of physiological and anatomical differences between germfree and conventional animals, the microbiota as "the most cell-rich biochemically active organ" of the human body.

- *Helmut Haenel and colleagues (German Democratic Republic).*[14] Culture-dependent studies of the fecal microbiota in childhood.
- *Tomotari Mitsuoka and colleagues (Japan).*[15] Innovative culture methods for anaerobic bacteria, comparisons of microbiota composition in humans and other animals, changes in microbiota composition with respect to aging.
- *Pierre Raibaud, Robert Ducluzeau, and colleagues (France).*[16] Innovative culture-dependent studies of commensal bacteria, gnotobiotic animal experimentation involving defined mixtures of cultivated bacterial species, the fecal microbiota in early life.
- *H. Williams Smith (UK).*[17] Comparative acquisition of the gut microbiota of farm animals using culture-dependent methods, relative abundances of bacterial groups present in various levels of the gastrointestinal tract.

The combined contributions of these clinicians and scientists led to the following conclusions about the gut microbiota:

- The microbiota of healthy adult humans is composed of bacteria that are able to live anaerobically.
- Hundreds of bacterial species make up the fecal microbiota; a small proportion of the inhabitants of the bowel are Archaea that produce methane.
- The distal small and large bowels contain diverse and abundant bacterial species.
- Transient bacteria ingested with food and inhabitants of the upper digestive tract can be detected in feces along with the colonic microbiota members.
- The colonic microbiota contains trillions of bacterial cells.
- The microbiota contributes to about 50% of fecal mass.
- A biological (ecological) succession occurs in the bowel during early life.
- Some commensals are associated with the mucus covering the bowel epithelium, at least in some animal species and conditions.
- The microbiota is regulated by host-associated factors (allogenic) and factors generated by the communities of bacteria themselves (autogenic).
- The gut microbiota is equivalent, biochemically, to an organ of the human body.

I am a beneficiary of the contributions of these giants, and have been fortunate to work in the area of gut commensals for several decades, mostly at the University of Otago. Other giants of bowel bacteriology will be mentioned in subsequent chapters. The reason for recording some of them here is because the 1960s were a long time ago – "before PubMed" – and we should not forget these brilliant investigators.

In ordering the chapters in this book, I wondered what would be best for the reader. The gut microbiota as a topic might appeal to popular science readers or undergraduates and graduates interested in gaining a little more microbiological knowledge without delving too much into technology. These readers would probably prefer a "good read." I hope that Chapters 2–10 logically flow from one to the other and satisfy this desire. Chapter 2 provides an overview of the human bowel microbiota and some of the methods that are used to investigate it. Chapter 3 considers the characteristics of microbial communities, and how and why we became hosts to so many different kinds of bacteria. Chapter 4 attempts

to describe how microbial communities are formed and the forces that drive the enrichment of particular kinds of bacteria. This leads to discussion in Chapter 5 of some ecological precepts that might define a bowel society. Studies concerning simulations of the human bowel in the laboratory, and what has been learned from them, are discussed in Chapter 6. Eavesdropping on the secret lives of microbes in the bowel is covered in Chapter 7, while the related topics of "biological Freudianism" and imbalances in the relative abundances of members of the microbiota in disease versus health ("dysbiosis") are dealt with in Chapters 8 and 9. Chapter 10 sums up the story and draws a roadmap to increased understanding of bowel bacteria. Finally, a brief technical addendum provides quick reference to analytical methods. Some of the chapters contain terms in **bold** text. These are defined at the end of the relevant chapter.

It has been difficult to write a book about such a fast-moving area of research as that of "bowel bacteria." My aim has to been to record conceptual progress in the field, rather than to review the literature. Reviews of bowel bacteria often appear in journals, are usually highly speculative, and focus heavily on observations from experimental animal research rather than on studies of humans. I do not think that there is really any "model" for humans, so I make limited use of rodent research, and include it only where it might help us understand the bowel bacteria of humans. I have been selective in the choice of citations – which can be considered indicative of an advance in knowledge – and apologize in advance to the many researchers who will feel slighted because I have not mentioned their admirable work.

My first foray into writing a book was the small volume, *Normal Microflora: An Introduction to Microbes Inhabiting the Human Body*,[18] which was based in part on lectures delivered to second-year microbiology students at Otago. That book was well received and is still available online. I hope that you will enjoy this one, too!

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2

Prime Facts

- *What is the human bowel microbiota?*
- *How can we learn about microbiota composition?*

An enormous appreciation of the collection of microbes, mostly bacteria, that inhabits the large bowel (colon) of humans in general good health has accrued during the past 15 years. Although much was known of this microbial collection even in the last quarter of the 20th century (Chapter 1), huge advances in nucleic acid sequencing and bioinformatics methodologies in the 21st have resulted in a much more detailed knowledge of the microbial component of the large-bowel ecosystem.[1] Admittedly, most of this knowledge has been obtained through the examination of fecal specimens. Feces tell us about the kinds of bacteria that are present in the digesta in the last part of the bowel (rectum), but nothing specifically about the other parts of the digestive tract.[2] It is usually assumed that the kinds of bacteria are similar throughout the colon, yet, as John Cummings and George Macfarlane have demonstrated, whether or not the microbiota composition is the same, the bacterial metabolic activity is markedly different proximally and distally.[3]

Total carbohydrate content of the digesta in the proximal colon (cecum) is about 20%, dropping to 11% in the distal colon (sigmoid-rectum). The reduction in carbohydrate content is due to the breakdown of carbohydrates by the microbiota. As Cummings and Macfarlane point out,[3] the digestive function of the colon principally involves the breakdown by bacteria of carbohydrates (fermentation) into short-chain fatty acids (SCFAs) under anaerobic conditions. The inflow to the colon is digesta that has already undergone digestion in the stomach and small bowel. Therefore, only materials that are nondigestible or nonabsorbable by human processes pass to the colon. There, the digesta is mixed, and is retained in the proximal region (cecum and right colon) for 6–12 hours (see Figure 2.1). The digesta then passes through the transverse colon to the left colon for storage and excretion.

It is estimated (based on UK inhabitants) that about 1.5 kg of digesta enters the colon each day, ultimately to be excreted as feces at 120 g per day. Digesta retention time within the whole human gut is about 60 hours. The major products of bacterial fermentations in the colon are the SCFAs acetic, propionic, and butyric acid (typically in the ratio 3 : 1 : 1; see Table 2.1), the gases hydrogen and carbon dioxide, lesser amounts of branched-chain SCFAs (originating from the

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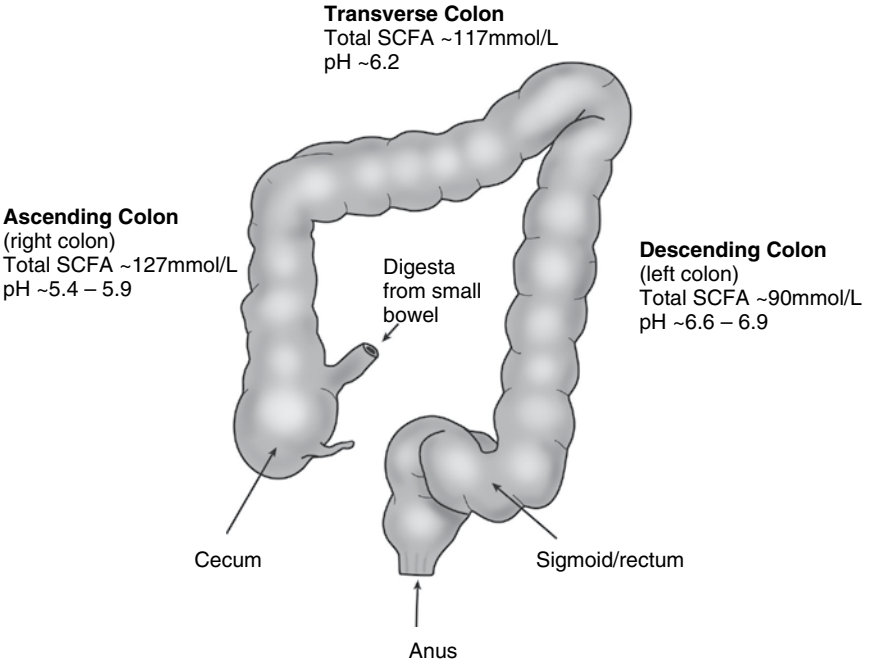

Figure 2.1 Anatomical regions of the large bowel (colon) of humans, showing associated pH and total SCFA concentrations. Source: Numerical data from Cummings and Macfarlane 1991.[3]

fermentation of amino acids), and ammonia, amines, and phenols. Methane is produced in some human colons.[4–6] SCFA concentrations are greatest in the cecum and right colon, and fall progressively towards the distal regions (Figure 2.1). pH is lowest in the cecum (~5.5) and highest in the rectum (~6.9), reflecting the bacterial fermentative production of acids. Thus, the greatest fermentative activity is associated with the highest availability of carbohydrate substrates, which seems logical. It is noteworthy that about 95% of the SCFA produced in the colon is absorbed by the bowel mucosa, so fecal SCFA concentrations do not directly reflect what is happening in this respect in the colon itself. To some extent, therefore, the real lives of microbes in the colon remain hidden to us. Even internal sampling methods do not help much. Invasive techniques (colonoscopy and collection of mucosal biopsies) that are capable of accessing specific bowel regions have interpretative problems associated with them.

Colonoscopy is an essential clinical procedure in assessing the condition of the colonic mucosa. For effective colonoscopy, the contents of the colon need to be washed from the organ, in a procedure termed "bowel cleansing." This is achieved by having the patient drink copious amounts of water, as well as chemicals that promote emptying of the colon. Bowel-cleansing preparations are broadly classified into three groups. (i) Osmotic laxatives are the most common: these include agents such as sodium phosphate, magnesium citrate, and mannitol, which increase colon water content by causing fluid to leave the mucosa (efflux) and enter the intestinal lumen. (ii) "Macrogols" are various forms of polyethylene